Claims

It is claimed:

1. A method of producing a transformed microorganism, comprising:
   (i) selecting a competent microorganism;
   (ii) producing a DNA construct *in vitro*; and
   (iii) directly transforming said microorganism with said DNA construct such that the DNA construct becomes integrated into a chromosome of said microorganism.

2. The method of claim 1, wherein said microorganism is selected from the group consisting of Acinetobacter, Thermus, Deinococcus, Radiodurans and Bacillus.

3. The method of claim 2, wherein said microorganism is a Bacillus.

4. The method of claim 3, wherein said Bacillus is a super-competent strain.

5. The method of claim 4, wherein said super-competent Bacillus is a Pxyl-comK strain.

6. The method of claim 1, wherein said DNA construct comprises homologous DNA selected from the group consisting of wild-type, mutagenized and modified DNA.

7. The method of claim 1, wherein said DNA construct comprises heterologous DNA selected from the group consisting of wild-type, mutagenized and modified DNA.

8. The method of claim 1, wherein said DNA construct comprises an incoming sequence sequence of interest, flanked on each side by a homology box.

9. The method of claim 8, wherein said DNA construct further comprises stuffer sequences.

10. The method of claim 1, wherein said DNA construct is a non-plasmid DNA construct.

11. The method of claim 1 wherein the DNA construct is produced without the use of a shuttle vector or an intermediate host.

12. The method of claim 1, further comprising the steps of selecting a target sequence in a chromosome of said competent microorganism, and increasing the homology between said target sequence and said DNA construct.

13. A library of mutants produced by the method of claim 1.

14. Directed evolution of a sequence in the host cell chromosome, comprising:

(i) *in vitro* mutagenesis of a DNA construct,

(ii) direct transformation of the mutagenized sequence into a competent host cell,

(iii) screening for, or selection of, mutants possessing or exhibiting a desired property, and

(iv) repeating steps (i)-(iii) for one or more rounds.

15. The method of claim 14 wherein the host cell is a Bacillus.

16. The method of claim 15 wherein the Bacillus is a Pxyl-comK strain.

17. The method of claim 14, carried out so as to evolve single-copy genes of a competent Bacillus strain.

18. A method for constructing a sequence of interest at a target sequence of a selected microorganism, wherein said target sequence includes a residing marker, said method comprising the steps of:

(i) assembling a DNA construct in vitro comprising an incoming sequence, a selectable marker, and two flanking sequences which are homologous to sequences of said target sequence, wherein said selectable marker of the DNA construct is different than the residing marker of the microorganism;

(ii) transforming said microorganism with the DNA construct under conditions permitting the incoming sequence and selectable marker to inactivate the residing marker, and selecting for transformants that include the selectable marker;

(iii) repeating steps (i) and (ii) wherein with each repetition of said steps the DNA construct comprises a selectable marker different from the selectable marker in the previous step and the selectable marker of said previous step acts as the residing marker in said microorganism.

19. The method of claim 18, further comprising, after step (ii), the step of:

testing the transformants for loss of the residing marker, thereby verifying that the construct was incorporated into the correct locus of the chromosome.